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HERBAL MICROSPHERES OF CROSSANDRA INFUNDIBULIFORMIS L FOR ENHANCED ANTIULCER ACTIVITY

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ABSTRACT

Herbal medicines have been shown to be less expensive and safer than synthetic pharmaceuticals in the treatment of many ailments, although they are less effective and not targeted. As a result, using novel drug delivery methods to administer herbal extracts can significantly improve their release, potency, and activity. Crossandra infundibuliformis L. leaves methanol extract had been combined into mucoadhesive microspheres. Sodium alginate, carbopol 934, and sodium CMC were used to make mucoadhesive microspheres (carboxy methyl cellulose). The produced microspheres were tested for physical and physicochemical characteristics, invitro release, and invivo antiulcer efficacy in Wistar albino rats with ethanol-induced ulceration. When compared to extract delivered directly, the produced microspheres demonstrated comparable physicochemical characteristics, prolonged release for up to 8 hours, and enhanced antiulcer efficacy. After adequate chemical and biological standardisation, herbal extracts can be given utilising new drug delivery methods.

Key words: Microspheres, Antiulcer Activity, Crossandra Methanol Extract, Standardization.

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INTRODUCTION

For many decades, medicines have been delivered to patients in the form of tablets, capsules, pills, creams, ointments, liquids, aerosols, injections, and suppositories as carriers to treat acute or chronic diseases. To maintain the minimal effective concentration of medication in the body, these formulations must be taken multiple times each day. As a result, medication levels fluctuate and unfavourable toxicity occurs. As a result, there is a rising interest in controlled drug delivery systems (Chien YW, 1992)

Given the unfavourable side effects of synthetic medicines, herbal remedies are regarded as the finest option accessible to cure any condition. (Avinash Kumar Reddy *et al., 2011*)

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There is a surge in recommendations for herbal medicines these days, which is increasing market need for a standardised product with predictable and repeatable performance. So, in order to enhance the action of herbal medicines, innovative drug delivery methods have been developed to administer standardised plant extracts. Many studies have been conducted in this regard in order to successfully use innovative medication delivery to herbal medicines in the process.

Various research have looked into lipid-based drug delivery systems and found that they have the ability to manage drug release and target medicines. Pharmacosomes improve the drug's biopharmaceutical characteristics, resulting in increased bioavailability. Phytosomes are new compounds made up of lipophilic complexes of plant extract that have been proven to be efficient in terms of medication delivery. These are enhanced versions of traditional herbal extracts with better pharmacokinetic and pharmacological characteristics that can be utilised to treat acute illnesses .(Kusum Devi V *et al.,* 2010) Phytosomes containing

phospholipid extracts of plants such as Silybum marianum, Ginkgo biloba, and Panax ginseng have been produced (Semalty A, 2009). Microcapsules containing entrapped herbal water-soluble extracts of Plantago major and Calendula officinalis L. (PCE) were shown to be beneficial in healing stomach ulcers via layer-by-layer adsorption of carrageenan and oligochitosan onto calcium carbonate micro particles (Borodina TN & Rumsh LD, 2008). Taking into account the benefits of drug delivery, the current study was carried out to incorporate the methanolic extract of the leaves of Crossandra infundibuliformis L. into mucoadhesive microcapsules of sodium alginate, carbopol 934, and sodium CMC and evaluate their physicochemical parameters and invivo pharmacological activity.

EXPERIMENTAL METHODS

Chemicals and Materials

The plant's fresh leaves were gathered from the hills of Tirumala, Andhra Pradesh, India, and certified by Dr. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, before being conserved in the laboratory as a herbarium specimen. All of the polymers and reagents were obtained from SD Fine Chem Ltd in Mumbai.

Laboratory Rats

Albino Wistar rats of both sexes weighing 170-190gms were chosen. They were kept in conventional polypropylene cages at ambient temperature (25°C) with 12-12hr dark and light cycles. They were given a standard pellet feed purchased from Hindusthan Ltd. in Bangalore, as well as unlimited water. Animals were denied food but not water for 24 hours before to the experiment.

Extraction

The plant leaves were dried in the shade before being crushed and extracted with methanol using a soxhelt device. The extract (18.52% w/w) was dried and utilised for future research.

Preformulation Method

The standard plot of MECL (Crossandra infundibuliformis L. Methanolic Extract) in 0.1 N HCl was drawn spectrophotometrically (UV-Visible-1700, Shimadzu spectrophotometer) at 271 nm as part of preformulation investigations, resulting in a straight line with r2 value 0.997. The extract and polymers were subjected to an FTIR examination to determine their compatibility.

Preparation of drug loaded microspheres

MECL (Crossandra infundibuliformis L. Methanolic Extract) loaded mucoadhesive microspheres were created using an orifice ionic gelation technique with Sodium alginate, Carbopol 934, and Sodium CMC (carboxy methyl cellulose). Briefly weighed amounts of polymers and MECL were dispersed in 10ml distilled water with continuous stirring at 300 rpm for 30 minutes. The resulting dispersion was added drop by drop into a 10% calcium chloride solution using a syringe (17 gauge) with a needle. The produced microspheres were left for 30 minutes to cure completely before being recovered by filtering through a sintered glass filter and drying in a hot air furnace at 500 degrees Celsius for 1 hour (Harborne JB, 1998; Wagner H & Bladt S, 1996).

Evaluation of microspheres

The evaluation of prepared microspheres was done as follows (Bhabani SN *et al.*, 2009)

Scanning Electron Microscopy (SEM)

SEM examination was performed on the surface morphology using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to inspection, samples were mounted on an aluminium stub using double-sided adhesive tape and electrically conductive by coating in vacuum with a thin layer of gold (about 20 nm). The scanning electron microscope was set to run at a 5 kV acceleration voltage and a resolution of 4000.

Drug entrapment (DE)

Powdered drug-loaded microspheres (100 mg) were suspended in 100 ml of 0.1N HCl solution. The resulting dispersion was maintained for 24 hours with constant agitation for full mixing before being filtered through a 0.45 m membrane filter. The drug content was calculated using the extract standard graph (r2 = 0.997). The equation was used to determine the drug entrapment (DE).

$$DE = (Pc / Tc) \times 100,$$

Where, Pc is practical content, Tc is the theoretical content.

% moisture loss

The extract-loaded microspheres were tested for percentage moisture loss, which provides insight into their hydrophilic nature. The weighted microspheres (W1) were originally stored in a desiccator containing calcium chloride at 370°C for 24 hours. They were weighed, and the process was repeated until no further change in sample weight was noticed. The total weight (W2) was recorded.

$$\text{Moisture loss} = [(W_1 - W_2)/ W1] \times 100.$$

Determination of swelling index

Microspheres of known weight were immersed in a dissolution solution (0.1N HCl) for 6 hours before being centrifuged. The wet weight of the swollen microspheres was determined by blotting the particles with filter paper to remove absorbed water on the surface and then weighing immediately on an electronic balance.

After that, the percentage swelling of microspheres in the dissolving medium was determined using the equation,

$$Sw = [(Wt- Wo)/Wo] \times 100$$

Where Sw denotes the percentage of swelling of the microspheres, Wt denotes the weight of the microspheres after swelling, and Wo denotes the starting weight of the microspheres.

Mucoadhesion test

The falling film method is one of the best ways for determining the mucoadhesion strength of mucoadhesive particle systems such as mucoadhesive microspheres and suspensions. A fixed number (N1) of microspheres (100 microspheres) (Rajeshwar Kamal Kant Arya *et al.,* 2010) were distributed over a fresh intestinal segment of sheep, placed on a slanted slide at an angle of 450, and incubated for 15 minutes. For 3 hours, a 0.1N HCl solution was gently poured over the segment at a flow rate of 1ml/min (Chirag N *et al.,* 2009). The effluent was collected on Whattman filter paper, and the number of detached microspheres (N2) was recorded. The equation was used to calculate the percentage of mucoadhesion.%

$$\text{Mucoadhesion} = N_1 - N_2/ N_1 \times 100$$

In vitro drug release testing

MECL release from microspheres was measured in vitro (González M *et al.,* 2003). The in vitro drug release research was carried out in a basket type dissolving test equipment according to USP standards. Microspheres were put in a basket of a dissolving tank containing 900ml of 0.1N HCl and agitated at 100 rpm. At 1 hour intervals, aliquots of samples (5 ml) were extracted and filtered using whatman filter paper. With the dissolving medium, the sink state was maintained.

Antiulcer activity comparison of microspheres

The microspheres were tested for antiulcer efficacy in Wistar Albino rats with Ethanol-induced gastric ulceration. Animals were divided into four groups: Group 1 (Negative control) received normal saline intramuscularly, Group 2 (Positive control) received standard drug, sucralfate suspension 100mg/kg intramuscularly, Group 3 received MECL microspheres intramuscularly, and Group 4 received MECL 400mg/kg intramuscularly. MECL microspheres were dosed to be equal to 400 mg/kg MECL (Biswas K *et al.,* 2003). Each group was subdivided into three groups: 2 hrs, 4 hrs, and 8 hrs, each with six animals, representing scarification of animals for calculating ulcer index at 2, 4, and 8 hrs following administration. The number of ulcers per stomach was counted, and the severity of the ulcers was graded microscopically (10X).

The ulcer index was calculated as a measure of antiulcer activity using the formula,

$$\text{Ulcer index}=UA+US+UP/10.,$$

Where UA is the average number of ulcers per animal, US is the ulcer severity score, and UP is the percentage of animals with ulcers. UP=Total number of ulcers in a group/total number of animals/100.

Statistical analysis

All statistical measures, including Mean, Standard Deviation (S.D.), Standard Error in Means (S.E.M.), and their sequential differences, were computed using one-way ANOVA followed by Dunnet's T-test at P0.001 level of significance.

RESULTS AND DISCUSSION

Individual FTIR investigations on Carbopol 934, Sodium CMC, Sodium alginate, MECL, and microspheres yielded the spectra illustrated in figure 2. MECL had a peak at 3435.76 cm-1, which corresponded to O—H stretching, and all of the polymers had a peak in a similar range. Interestingly, in MECL microspheres, this peak shifts to 3420.08 cm-1, indicating the establishment of hydrogen bonds between the extract and polymers. The extract's peak at 1651.34 cm-1, indicating the C=O functional group, has been moved to 1622.41 cm-1, confirming the hypothesis of hydrogen bonding between the oxygen atoms from the C=O group in the extract and the hydrogen atoms of the polymers' OH. There were no further peaks in the spectra of MECL microspheres, suggesting that no new chemical compounds were formed, confirming that no chemical interaction had happened.

Following the table 2, five formulations had been developed and assessed. The percentage yield, drug entrapment, moisture loss, muco-adhesion strength, swelling index, in vitro drug release, and in vivo antiulcer efficacy of prepared microspheres were all evaluated and tabulated. All of the formulations' percentage yields were tested, and formulation F2 had the greatest percentage yield of 93 percent, followed by F3 at 90 percent, which was also considerably comparable to F2. In contrast, the drug entrapment efficiency of formulation F3 outperforms that of formulation F2.

SEM was used to assess the particle shape and surface morphology of the produced microspheres. Figure 3 depicts the pictures. Each microsphere is roughly 700m in diameter on average. It is approximately spherical in form and with a smooth surface. Microspheres were dried adequately as seen from the absence of fractures and folds on the surface. Microsphere sectioning revealed an equal distribution of extract. The swelling indices of formulations with a high carbopol to CMC ratio were higher than those with a low ratio. This implies that the carbopol is responsible for absorption water and swelling. It is apparent from F5 having the greatest swelling index of 77 percent, which had a high carbopol content, and as predicted, F3 should have the lowest swelling index. However, F4 has swollen less, which might be owing to

the low concentration of sodium alginate. This lends credence to the anti-inflammation action of sodium alginate. Moisture loss % readings of all the formulations are under limitations. Formulation F3 had a lower value than the other formulations. So it can be confirmed that the formulation is adequately dried, and the quantity of moisture present in the microspheres is also affected by the carbopol concentration, as evidenced by their swelling indices.

The falling film method was used to perform the in vitro muco-adhesion test. F3 had the highest muco-adhesive strength with a value of 89, followed by F2 with a value of 86. Formulation F5 yielded the lowest value. All of the data indicate that the presence of Sodium CMC has an effect on mucoadhesion. The higher the sodium CMC concentration, the greater the muco-adhesion. However, sodium alginate has a good muco-adhesion property, which can be proven when formulations F1 and F5 are compared. The quantity of sodium CMC remained constant with changing concentrations of sodium alginate, indicating that F1 has a superior adhesion. Sodium alginate is a popular encapsulating ingredient that aids sodium CMC in muco-adhesion. This implies that the kind of polymer employed in the production of microspheres, as well as their concentration, impacts their capacity to adhere to mucosa. Table 2 shows that all formulations had good muco-adhesion strength overall.

Drug release studies in vitro

The drug release experiments were carried out, and the % was determined using a standard graph of quercetin. All formulations demonstrated well regulated release, with values ranging between 53.960.59 and 68.500.59 for formulas F2 and F3. Individually, the drug release rates for F1, F2, F3, F4, and F5 are 64.750.26, 53.960.56, 68.500.59, 54.960.56, and 65.910.23 in 8 hours. Formulations F1 and F3 demonstrated a sustained release. The release was projected to last another four hours. By integrating the methanolic extract inside the muco-adhesive microcapsules, the drug release can be maintained for up to 12 hours.

Antiulcer screening in vivo

At $p<0.001$, all of the values were significant to control. MECL microspheres showed less activity at the end of the second hour, but continued to compete with the other groups until the fourth hour. MECL in the fourth hour was competitive and effectively decreased ulcers when compared to other groups, with an ulcer index of 21.850.83. Until the fourth hour, all of the data are significant when compared to the control and standard. Interestingly, at the conclusion of the 8th hour, MECL microspheres exhibited a substantial reduction in ulcer index, 18.640.75, as compared to standard and MECL of 29.590.92 and 28.750.27, respectively. However, the activity of standard and MECL was halted at the fourth hour because to the possibility that they had progressed to the intestine due to stomach transit. This supports the hypothesis that MECL mucoadhesive microspheres remained in the stomach until the 8th hour, remaining intact with gastric mucosa and therefore decreasing ethanol-induced gastric ulcers. This opens up the possibility of further study into the use of NDDS to herbal medicines in order to demonstrate sustained and improved activity.

Table 1 Preparation of microspheres of Crossandra infundibuliformis L

Form.	*Sodium CMC (mg)*	*Carbopol 934 (mg)*	*Sodium alginate (mg)*
F1	50	50	200
F2	25	75	200
F3	75	25	200
F4	100	50	150
F5	50	100	150

400mg of extract was used in all the formulations

Table 2. Physicochemical parameters of mucoadhesive microspheres

Form. no.	*% yield*	*Moisture loss*	*Swelling index*	*mucoadhesion*	*Drug entrapment*
F1	72.47±0.75	5.56±0.95	68.35±0.48	84.61	94.89±0.05
F2	92.75±0.58	7.21±0.54	71.98±0.67	86.13	95.79±0.94
F3	91.65±0.98	5.01±0.83	70.68±0.46	89.54	97.01±0.66
F4	85.84±0.26	5.81±1.08	52.24± 0.91	85.81	93.76±0.44
F5	83.21±0.13	9.02±0.97	76.38±0.12	81.77	94.49±0.13

Values expressed as mean±Std deviation where n=3

Table 3. % drug release of extract loaded microspheres

Time in hrs	% drug release				
	F1	F2	F3	F4	F5
1	47.19±0.52	41.60±0.27	49.81±0.28	42.66±0.27	49.59±0.27
2	51.29±0.43	47.96±0.19	51.42±0.27	46.56±0.26	54.26±0.62
3	56.89±0.44	49.46±0.24	59.51±0.12	49.06±0.64	56.40±0.49
4	59.32±0.48	50.90±0.26	62.56±0.23	50.29±0.20	59.14±0.52
5	61.16±0.13	51.56±0.28	64.16±0.28	52.90±0.08	62.50±0.27
6	62.51±0.43	52.80±0.23	65.81±0.26	52.90±0.62	62.96±0.65
7	63.41±0.04	53.20±0.67	66.50±0.27	51.60±0.14	64.22±0.24
8	64.75±0.26	53.96±0.56	68.50±0.59	54.96±0.56	65.91±0.23

Values expressed as mean±Std deviation where n=3

Table 4. Anti-ulcer activity comparison of microspheres

Sl.	Group	Ulcer indices		
		2 hrs	4 hrs	8 hrs
1	Control	45.74±1.85	52.75±1.86	60.02±0.82
2	MECL 400 mg/kg	24.17±0.66*	20.97±0.75*	29.59±0.92* [a]
3	MECL microspheres	29.16±0.24*	21.85±0.83*	18.64±0.75*
4	Standard	20.02±1.21*	25.54±0.27*	28.75±0.27*[a]

Values expressed as mean±Std deviation where n=6

*P<0.001, significant compared to control; a P<0.001, significant compared to MECL microspheres.

Figure 1. FTIR spectra. A.MECL; B. Microspheres.

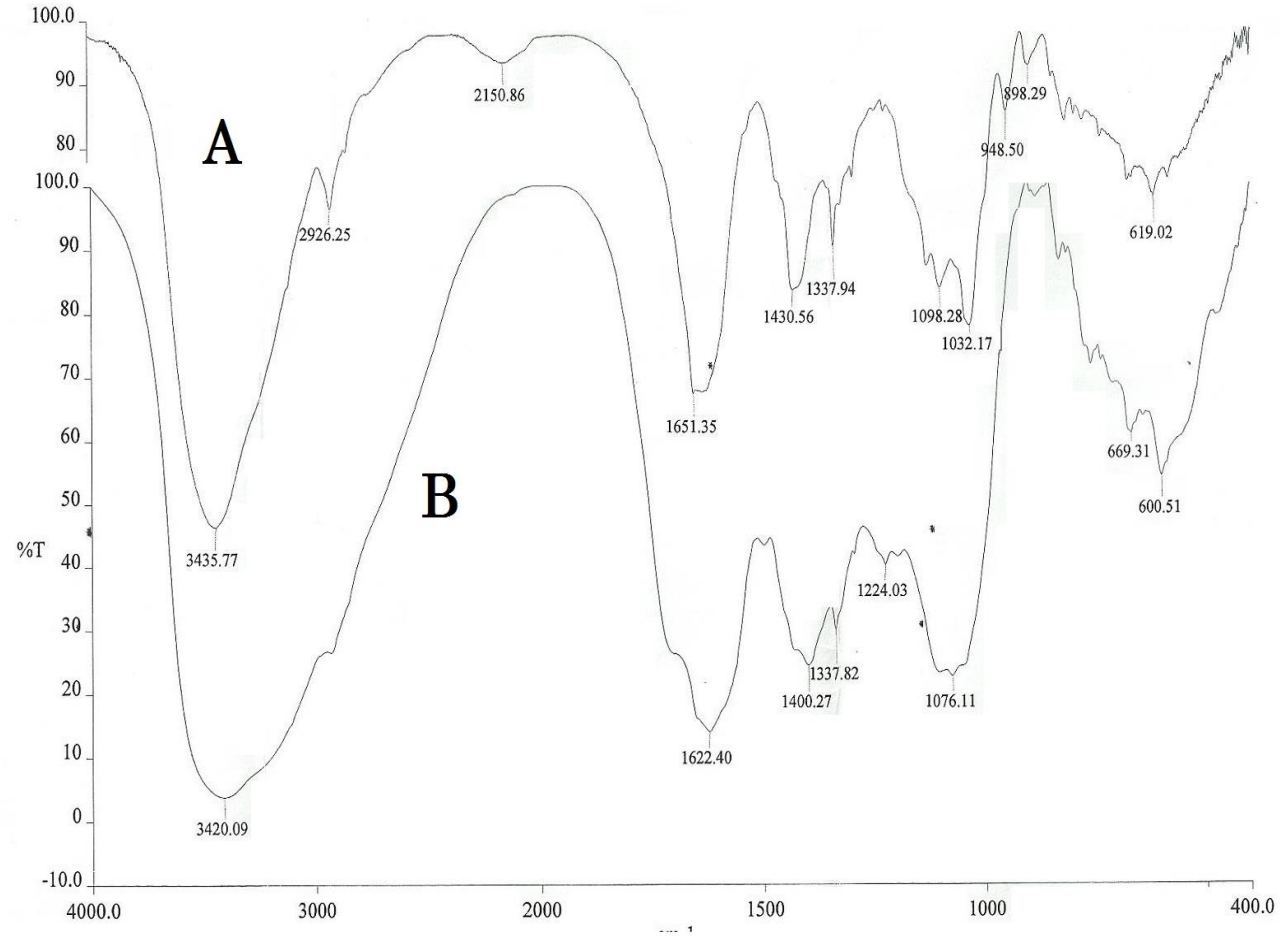


Figure 2. SEM Photographs of microspheres

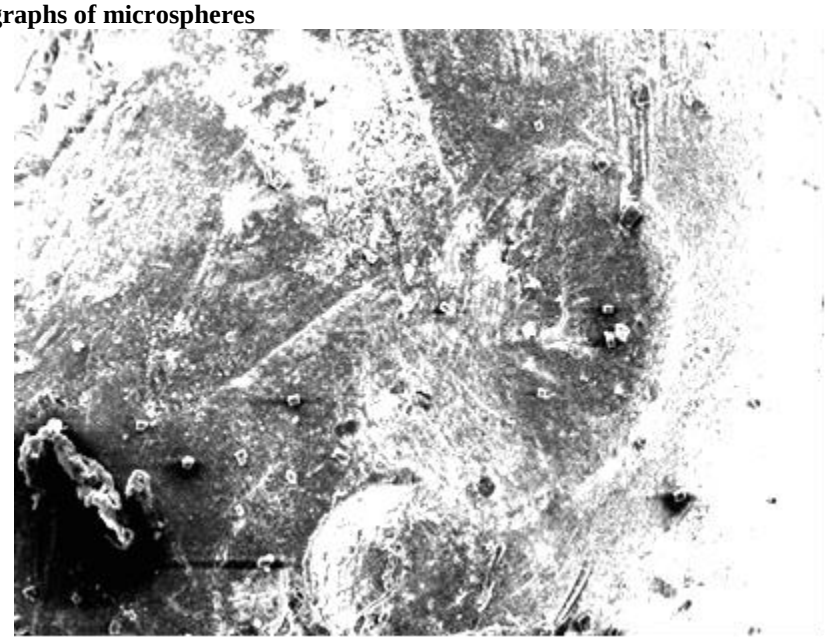
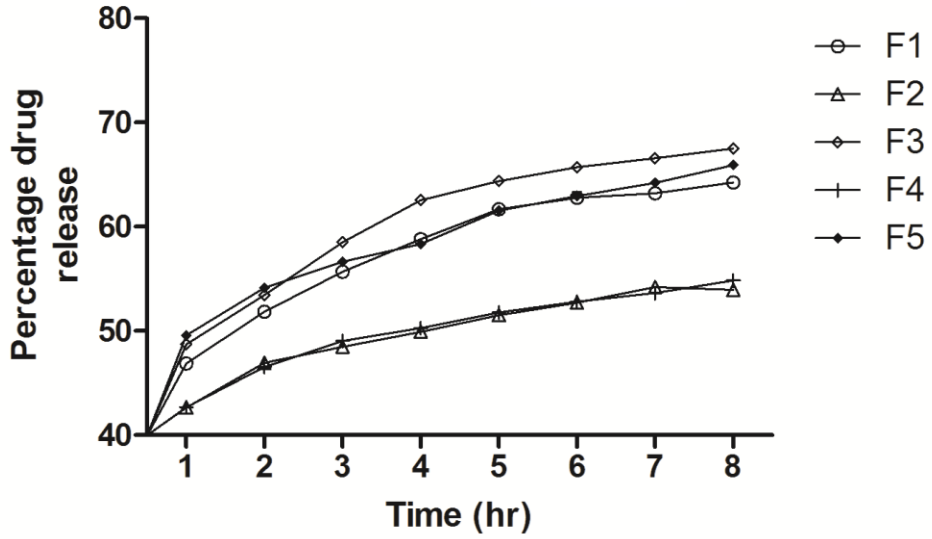


Figure 3. Drug Release from MECL Microspheres



CONCLUSION

Given the limitations of herbal formulations, including plant extracts into polymers and using NDDS for their administration should improve the quality of medicine for any condition. The incorporation of herbal extracts into muco-adhesive microspheres for the treatment of illnesses, particularly stomach ulcers, is a significant step forward for research in the field of gastro retentive drug delivery systems aimed at delivering herbal medicines. This might result in formulations that fulfil

patient compliance while also providing prolonged release and improved effectiveness while reducing adverse effects. The standardisation of herbal medications designed for NDDS, on the other hand, is the most important problem to address. The rate limiting step for research in this area is proper chemo-profiling and minimising pesticide and heavy metal toxicity.

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COMPETING INTERESTS

Authors declare there are no competing interests

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